Denaturation Transition of Stretched DNA

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We generalize the Poland-Scheraga model to consider DNA denaturation in the presence of an external stretching force. We demonstrate the existence of a force-induced DNA denaturation transition and obtain the temperature-force phase diagram. The transition is determined by the loop exponent c for which we find the new value $c = 4\nu - 1/2$ such that the transition is second order with $c = 1.85 < 2$ in $d = 3$. We show that a finite stretching force F destabilizes DNA, corresponding to a lower melting temperature $T(F)$, in agreement with single-molecule DNA stretching experiments.

Under physiological conditions the thermodynamically stable configuration of DNA is the Watson-Crick double helix. The constituent monomers of each helix, the nucleotides A, T, G, C, pair with those of the complementary helix according to the key-lock principle, such that only the base-pairs (bps) AT and GC can form [1]. Upon heating or titration with acid or alkali of double-stranded DNA, regions of unbound bps proliferate along the DNA until full separation of the two DNA strands at the melting temperature T_m; depending on the relative content of AT bps, T_m ranges between some 60 to 110°C [2]. The classical Poland-Scheraga (PS) model views DNA as an alternating sequence of intact double-helical and denatured, single-stranded domains (bubbles or loops). Double-helical regions are dominated by the hydrogen bonding of bps as well as base stacking, bubbles by the entropy gain on disruption of bps [3]. The PS model is fundamental in biological physics and has been progressively refined to obtain a quantitative understanding of the DNA melting process [4, 5]. DNA denaturation can also be induced mechanically, by longitudinal stretching of single DNA molecules by optical or magnetic tweezers or atomic force microscopes [6, 7, 8, 9]. At the transition, the plot of stretching force F versus mean DNA extension L exhibits a plateau at 60-90 pN [10, 11]. Force-induced destabilization of DNA has become a valuable tool, e.g., to probe the interaction of proteins that specifically bind to single-stranded DNA, at physiological melting temperatures $T_m(F)$ well below $T_m(0)$ of free DNA [12].

In this Letter we consider the force-assisted denaturation transition of double-stranded DNA in the framework of the PS model (Fig. 1). The thermodynamic state of the DNA molecule now depends on both temperature T and stretching force F. We find a bounded region of bound states in the (T, F) plane (Fig. 2). The shape of the transition line implies that finite stretching forces F indeed lower the melting temperature $T_m(F)$, and the calculated force-extension relations $F(L)$ exhibit a plateau over a certain range of DNA extension L (Fig. 3). Both observations are in agreement with force-induced
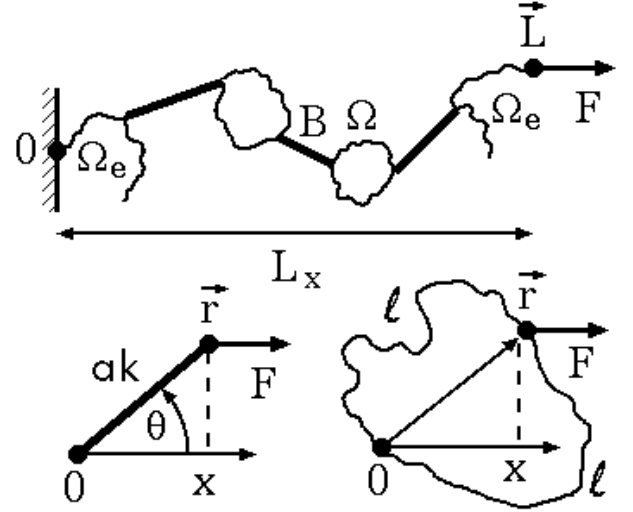

FIG. 1: Stretched DNA in the PS model with bound segments B and denatured loops Ω. The DNA is attached between $\mathcal{O}$ and $\mathbf{L}$ and subject to the stretching force F in x-direction. Perfect matching in heterogeneous DNA requires both arches of a loop to have equal length ℓ.

DNA melting experiments.

We treat the chain in the grand canonical ensemble in which the total number N of bps and the end-to-end vector $\mathbf{L}$ fluctuate. The partition function in $d = 3$ becomes

$$\mathcal{Z}(z, F) = \sum_{N=1}^{\infty} \int d^3L \, \mathcal{Z}_{\text{can}}(N, \mathbf{L}) z^N \exp(\beta F L_x) \quad (1)$$

with $\beta = 1/(k_B T)$. $\mathcal{Z}_{\text{can}}(N, \mathbf{L})$ is the canonical partition function of a chain of N bps with fixed end-to-end vector $\mathbf{L}$ and z is the fugacity. We assume that the force F acts in the positive x-direction, and L_x is the x-component of $\mathbf{L}$ (Fig. 1). If bound segments and bubbles are independent, $\mathcal{Z}$ factorizes:

$$\mathcal{Z}(z, F) = \Omega_e + \Omega_e \left\{ \sum_{n=0}^{\infty} [B\Omega]^n \right\} B\Omega_e \,, \quad (2)$$

the last term equaling $\Omega_e^2 B/(1 - B\Omega)$. The alternating sequence of bound segments and bubbles with weights B and Ω in Eq. (2) is complemented by the weight Ω_e of an open end unit at both ends of the chain. Note that only one strand of the end unit is bound to the, say, magnetic bead, while the other strand is moving freely.

We model a *bound segment* with $k = 1, 2, \ldots$ bps as a rigid rod of length ak where $a = 0.34$ nm is the length of a bound bp in B-DNA [10]. For simplicity we assume that the binding energy $E_0 < 0$ per bp is the same for all bps. The statistical weight of a segment with fixed number k and fixed orientation is then ω^k with $\omega = \exp(\beta\varepsilon)$ and $\varepsilon = -E_0 > 0$. Assuming that k fluctuates with fixed fugacity z, and rotates around one end while subject to the force F (Fig. 1), the statistical weight of the segment for fixed z and F becomes

$$B(z, \omega, F) = \sum_{k=1}^{\infty} \frac{(\omega z)^k}{4\pi} \int_{\Omega} d\Omega \, \exp(\beta F x) \qquad (3a)$$

$$= \frac{1}{2y} \ln\left(\frac{1 - \omega z e^{-y}}{1 - \omega z e^{y}}\right), \quad y \equiv \beta F a. \quad (3b)$$

Integration in Eq. (3a) is over the unit sphere with area 4π, and $x = ak\cos\theta$ where θ is the polar angle between segment and x-axis. At $F = 0$, $B(z, \omega, 0) = \omega z/(1 - \omega z)$ as found previously for the denaturation transition of free DNA [13]. Note that B is only well-defined for $\omega z e^y < 1$; in what follows we assume $z < e^{-y}/\omega$.

Denatured loops are considered as closed random walks with 2ℓ monomers, corresponding to ℓ broken bps. This loop starts at $\mathcal{O}$ and visits the point $\mathbf{r}$ after ℓ monomers (Fig. 1). The number of configurations of a loop is

$$\Omega(\ell, \mathbf{r}) = C_0(2\ell) p_\ell(\mathbf{r}) \qquad (4)$$

under this constraint, where $C_0(2\ell)$ counts the configurations of a loop of length 2ℓ starting at $\mathcal{O}$ and $p_\ell(\mathbf{r})$ is the probability that the loop visits $\mathbf{r}$ after ℓ monomers. For an ideal random walk in $d = 3$, $C_0(2\ell) \sim \mu^{2\ell}\ell^{-3/2}$ (μ is the connectivity constant) and $p_\ell(\mathbf{r}) \sim \mathcal{R}^{-3}\exp[-\lambda(r/\mathcal{R})^2]$ where $\lambda > 0$, $r = |\mathbf{r}|$, and $\mathcal{R} = b\ell^{1/2}$ is the scaling length of the walk. The amplitude b is proportional to the persistence length of the walk. Thus, $\Omega(\ell, \mathbf{r}) \sim s^\ell \ell^{-3} \exp[-\lambda(r/\mathcal{R})^2]$ where $s = \mu^2$. We assume that $\mathbf{r}$ moves freely and is subject to the force F in the positive x-direction. The weight of an ideal random loop for fixed ℓ and F is given by the Gaussian integral

$$\Omega(\ell, F) = \int d^3 r \, \Omega(\ell, \mathbf{r}) e^{\beta F x} = A s^\ell \ell^{-c} \exp(\alpha y^2 \ell) \quad (5)$$

where A is an amplitude, $c = 3/2$, and $\alpha = b^2/(4\lambda a^2)$. Finally, we sum $\Omega(\ell, F)$ over ℓ with weight z^ℓ to obtain the statistical weight for an ideal random loop

$$\Omega(z, F) = A \sum_{\ell=1}^{\infty} u^\ell \ell^{-c} = A\mathrm{Li}_c(u), \; u = sz\exp(\alpha y^2). \quad (6)$$

$\mathrm{Li}_c(u) = \sum_{\ell=1}^{\infty} u^\ell \ell^{-c}$ is the polylog function [14], converging for $|u| < 1$ for any c. For $u = 1$ three cases exist: (i) $c \leq 1$: $\mathrm{Li}_c(1)$ diverges; (ii) $1 < c \leq 2$: $\mathrm{Li}_c(1)$ converges but $\mathrm{Li}'_c(u)\big|_{u=1}$ diverges; (iii) $c > 2$: Both $\mathrm{Li}_c(1)$ and $\mathrm{Li}'_c(u)\big|_{u=1}$ converge. The limit $u = 1$ corresponds to the value $z_m(F) = \exp(-\alpha y^2)/s$ of the fugacity; thus, $\Omega(z, F)$ is only well-defined for $z \leq z_m(F)$ and diverges for $z > z_m(F)$. The statistical weight Ω_e of an end unit modeled as ideal random walk may be derived in a similar way and one obtains $\Omega_e(z, F) = A_e \mathrm{Li}_0(u)$.

For free DNA it was found that the nature of the denaturation transition is determined by the analytic behavior of $\mathrm{Li}_c(u)$ at $u = 1$: for $c \leq 1$ there is no phase transition in the thermodynamic sense; for $1 < c \leq 2$ the transition is second order, and for $c > 2$ it is first order [3, 13]. One finds $c = 3/2 < 2$ if the loops are ideal random walks. Self-avoiding interactions within a loop modify this value to $c = 3\nu = 1.76$ with $\nu = 0.588$ in $d = 3$ [15]. In both cases the transition is second order. Self-avoiding interactions between denatured loops and the rest of the chain were found to produce $c = 2.12 > 2$, driving the transition to first order [13, 16]. These results suggest that the inclusion of self-avoiding interactions generally shifts the loop exponent c to larger values, possibly effecting a change of the transition from second to first order.

To see how c changes when self-avoiding interactions within a loop are included for the case $F > 0$, we obtain the weights Ω and Ω_e for a self-avoiding walk for $\ell \to \infty$. Then, Eq. (4) holds with $C_0(2\ell) \sim \mu^{2\ell}\ell^{-d\nu}$ being the number of self-avoiding loops with 2ℓ monomers. The probability density $p_\ell(\mathbf{r})$ scales as $p_\ell(\mathbf{r}) = \mathcal{R}^{-d} g(r/\mathcal{R})$ where $\mathcal{R} = b\ell^\nu$ is the scaling length of a self-avoiding walk and $g(x)$ a scaling function. The function $g(x)$ is not known for a self-avoiding loop. In what follows we assume $g(x) \sim x^\phi \exp[-\lambda x^\delta]$ for $x \to \infty$ where $\lambda > 0$, ϕ is an exponent, and $\delta = 1/(1-\nu)$ is determined by an argument by Fisher [15]. This form of $g(x)$ is consistent with $p_\ell(\mathbf{r})$ for a Gaussian loop ($\nu = 1/2$, $\phi = 0$) obtained above. For the related *linear* self-avoiding walk starting at $\mathcal{O}$ and ending at $\mathbf{r}$ after ℓ monomers, the above form of $g(x)$ also holds and ϕ can be expressed in terms of known exponents [15, 17]. For the present case of a self-avoiding loop $\delta = 1/(1-\nu)$ still holds but ϕ is unknown. However, we will see that ϕ drops out from the result for $\Omega(z, F)$ in the limit $\ell \to \infty$ at $F > 0$. The integral in Eq. (5) is no longer Gaussian, but can be evaluated using the steepest descent method at $\kappa = \beta F b \ell^\nu \to \infty$. It turns out that in this limit the integral is dominated by values $r/\ell^\nu \to \infty$. With the above behavior of $g(x)$ at $x \to \infty$ we find for a self-avoiding loop [cf. Eq. (5)]

$$\Omega(\ell, F) = A s^\ell \ell^{-c} y^{1/(2\nu)-1} \exp\left(\alpha y^{1/\nu}\ell\right) \qquad (7)$$

for $\kappa \to \infty$ with the new loop exponent in $d = 3$,

$$c = 4\nu - 1/2 = 1.85 \; . \qquad (8)$$

FIG. 3: Force-extension curves $f = Fa/\varepsilon$ as function of $l = \langle L_x \rangle/(a\langle N \rangle)$ at fixed $t_1 < t_2 < t_3 < t_4$ (cf. Fig. 2b). Open circles mark second-order transitions ($c = 1.85 < 2$). Inset: $f(l)$ for $c = 2.5 > 2$ where the transition is first order.
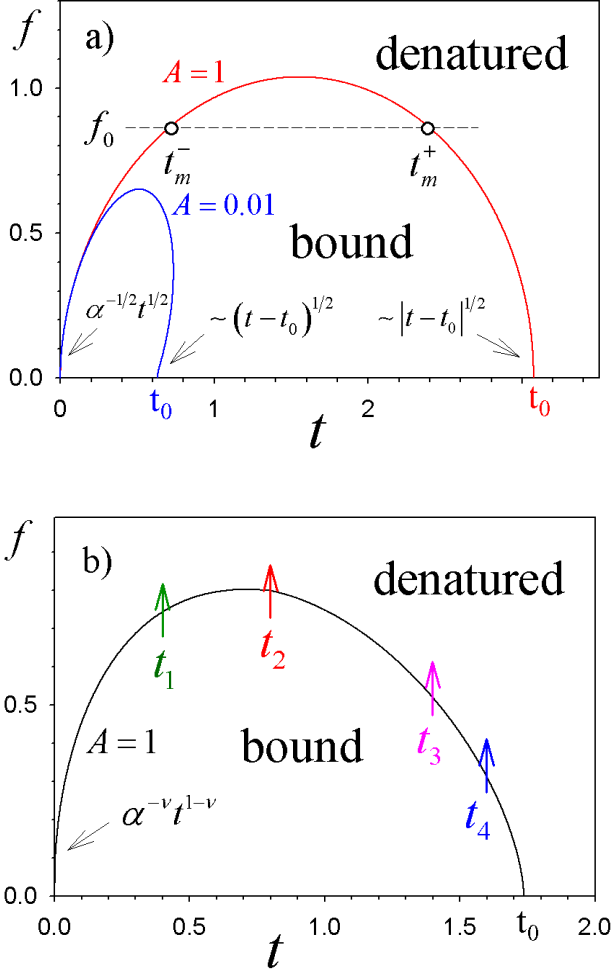

FIG. 2: Transition lines $f_m = F_m a/\varepsilon$ as function of $t = k_B T/\varepsilon$ for $\alpha = 1$, $s = 5$ for denatured loops modeled as (a) ideal random walks and (b) self-avoiding walks (cf. Fig. 3).

Thus, with self-avoiding interactions within a denatured loop and $F > 0$ the transition remains second order, but moves closer to first order compared to free DNA (with $c = 3\nu = 1.76$ obtained within the same approach). The amplitude A in Eq. (7) is proportional to the cooperativity parameter $\sigma_0 \ll 1$ quantifying the initiation of a loop in a previously intact double strand in the PS model [4, 5], such that also $A \ll 1$. Moreover, $\alpha = 0.6 \ldots 1.7 \approx 1$ using $\alpha = b^2/(4\lambda a^2)$ obtained for an ideal random walk where $b^2/\lambda = 2 L_p x_{ss}/3$; here, $x_{ss} = 0.6$ nm is the length of a base in single-stranded DNA [10] and values for the persistence length L_p for single-stranded DNA were found to range between 0.7 nm [6] and 2 nm [18].

Finally, we sum $\Omega(\ell, F)$ over ℓ with weight z^ℓ to obtain the statistical weight for a self-avoiding loop [cf. Eq. (6)],

$$\Omega(z, F) = A y^{-\theta} \text{Li}_c \left[sz \exp(\alpha y^{1/\nu}) \right], \quad (9)$$

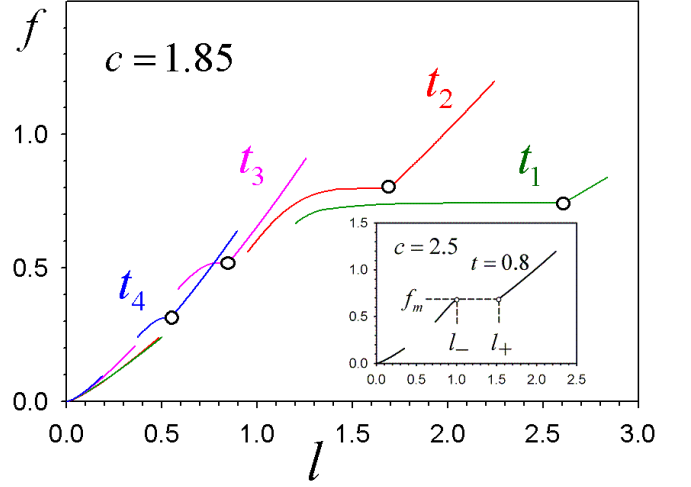where $\theta = 1 - 1/(2\nu) = 0.15$ in $d = 3$. The critical fugacity is now given by $z_m(F) = \exp(-\alpha y^{1/\nu})/s$. The weight $\Omega_e(z, F)$ for an end unit obtains similarly, the result being Eq. (9) with c replaced by $\zeta = 3/2 + \nu - 2\gamma = -0.232$, using $\gamma = 1.16$.

Phase diagram. We now obtain the transition line between bound and denatured states in the (T, F)-plane in the thermodynamic limit $N \to \infty$. For given fugacity z the average number of bps (open and closed) becomes

$$\langle N \rangle = \partial \ln \mathcal{Z}(z, \omega, F)/\partial \ln z, \quad (10)$$

where we explicitly include the argument ω from Eq. (3) in the partition function (2). If N is set one has to choose a fugacity z such that $N = \langle N \rangle$; in this case z becomes a function of ω, F, and N. We denote the value of z in the limit $N \to \infty$ by $z^*(\omega, F) \equiv \lim_{N \to \infty} z(\omega, F, N)$. Similar to the case $F = 0$ [13], $z^*(\omega, F)$ is the lowest value of z for which expression (10) diverges. In the bound state the divergence turns out to occur when the denominator in $\Omega_e^2 B/(1 - B\Omega)$ vanishes [see text below Eq. (2)], implying $z^*(\omega, F)$ to satisfy

$$B[z^*, \omega, F]\Omega[z^*, F] = 1, \quad \text{bound state}. \quad (11)$$

Conversely, in the denatured state the divergence occurs because $\partial_z \Omega_e(z, F)$ diverges, which implies

$$z^*(\omega, F) = z_m(F), \quad \text{denatured state}, \quad (12)$$

where $z_m(F)$ is the critical fugacity obtained above (which is independent of ω). Thus, starting in a bound state in the (T, F)-plane and approaching the transition line by varying T and F, the value $z^*(\omega, F)$ is determined by Eq. (11) and increases until it reaches the value $z_m(F)$ from Eq. (12). At this point the denaturation transition occurs. In the denatured state $z^*(\omega, F)$ is given by

Eq. (12). Right at the transition both Eqs. (11) and (12) hold simultaneously. Using $\Omega[z_m(F), F] = Ay^{-\theta}\text{Li}_c(1)$ by definition of $z_m(F)$ this implies $A(\beta Fa)^{-\theta}\text{Li}_c(1) = 1/B[z_m(F), \omega, F]$, relating F and ω, or, equivalently, the reduced force $f = Fa/\varepsilon$ and temperature $t = k_B T/\varepsilon$, for the transition line in the (t, f)-plane.

The shape of the transition line $f_m(t)$ depends on A, α, and s. Fig. 2a shows $f_m(t)$ for $A = 1$, $\alpha = 1$, and $s = 5$ for the case that denatured loops are ideal random walks ($\theta = 0$, $\nu = 1/2$). The transition line for the more realistic value $A \ll 1$ is also shown (here $A = 0.01$). The line $f_m(t)$ separates a finite region of bound states from an infinite region of denatured states. The point $(t_0, f = 0)$ with $t_0 = t_m(f = 0)$ corresponds to the traditional melting transition for free DNA ($F = 0$). The line $f_m(t)$ for $A = 1$ contains a region in which $f_m(t)$ *decreases* with t, such that increased stretching forces f lower the melting temperature $t_m(f)$, corresponding to force-induced destabilization of DNA [10]. Interestingly, for $A = 0.01$, application of a small stretching force f first *increases* t_m [19, 20]. Moreover, $f_m(t)$ vanishes for both $t \to t_0$ (as $|t - t_0|^{1/2}$) and $t \to 0$ (as $\alpha^{-1/2}t^{1/2}$). This means that for given $0 < f_0 < f_{\max}$, where $f_{\max}$ is the maximum of $f_m(t)$, the chain does not only denature at a large $t_m^+(f_0)$ but also at a small $t_m^-(f_0)$, as indicated in [21]. This behavior can be traced back to a balance of the terms $(\beta Fa)^2$ and βFa in $z_m(F) = \exp(-\alpha y^2)/s$ and Eq. (3b), respectively [22]. For $(\beta Fa)^2 \ll \beta Fa$, i.e., $k_B T \gg Fa$, the melting transition at $t_m^+(f_0)$ is mainly driven by the entropy gain on creation of fluctuating loops, similar as for free DNA. For $k_B T \ll Fa$ the transition at $t_m^-(f_0)$ is due to the fact that $B[z_m(F), \omega, F]$ *decreases* with $y = \beta Fa = f/t$ in the denatured state, due to the rapid decay of $z_m(F)$ [cf. Eq. (3b)] [23]. Fig. 2b shows the line $f_m(t)$ for self-avoiding loops with $A = 1$ and $c = 1.85$. Note that Eq. (9) reduces to the known result for a free self-avoiding loop ($y = 0$) only if $\theta = 0.15$ is replaced by $\theta = 0$; this is not a contradiction since Eq. (9) is based on the assumption that $\kappa = \beta bF\ell^\nu$ is large. To include in Fig. 2b the behavior of $f_m(t)$ for $f = Fa/\varepsilon \to 0$ we use Eq. (9) with $\theta = 0.15$ for $y > 1$ and $\theta = 0$ for $y \leq 1$.

Force-extension relations. In thermal denaturation of DNA one measures the fraction Θ of bound bps as function of T. From the partition function (1) the average number $\langle M \rangle$ of bound bps is $\langle M \rangle = \partial \ln \mathcal{Z}/\partial \ln \omega$ and $\Theta = \langle M \rangle / \langle N \rangle$ with $\langle N \rangle$ from Eq. (10). Conversely, stretching experiments on DNA reveal its response to an applied mechanical stress. The mean of the component of the DNA extension along F is $\langle L_x \rangle = \beta^{-1}\partial \ln \mathcal{Z}/\partial F$. The average extension per bp in units of the bp-bp distance a is $l = \langle L_x \rangle/(a\langle N \rangle)$. For comparison with experiments and simulations we calculate l in the thermodynamic limit $\langle N \rangle \to \infty$. Consider the Gibbs-Duhem relation for the thermodynamic potential $\ln \mathcal{Z}(z, \omega, F)$ [24]: $N d\ln z + M d\ln \omega + \beta L_x dF = 0$. If N is fixed one obtains $d\ln z + \Theta d\ln \omega + l dy = 0$ where z is a function of ω, F, and

N. For $N \to \infty$, $d\ln z^* + \Theta d\ln \omega + l dy = 0$, $z^*(\omega, F)$ being the fugacity for $\langle N \rangle \to \infty$ as discussed above; $\Theta(\omega, F)$ and $l(\omega, F)$ are the bound bp fraction and reduced DNA extension in the same limit. For constant $y = \beta Fa$ (or $y = 0$) one finds $\Theta = -\partial \ln z^*(\omega, F)/\partial \ln \omega$ (so that $\Theta = 0$ in the denatured state due to Eq. (12) as expected). For constant ω, corresponding to constant $t = k_B T/\varepsilon$, we find $l(\omega, F) = -(a\beta)^{-1}\partial \ln z^*(\omega, F)/\partial F$. Based on this result Fig. 3 shows force-extension relations $f(l)$ at fixed $t_1 < t_2 < t_3 < t_4$ for the case that denatured loops are self-avoiding random walks (cf. Fig. 2b). The two sets of curves correspond to expansions of $f(l)$ for small f and close to the transition, respectively. The curves $f(l)$ display flattened regions close to the transition, in qualitative agreement with experimental force-extension relations for DNA. These regions become less pronounced as t increases and vanish for $t \to t_0 = t_m(F = 0)$. A force-extension relation for the case $c > 2$, for which the transition is first order, is also shown (here $c = 2.5$). In this case $l(f)$ jumps discontinuously from a value l_- to a larger value l_+ at the transition.

We have shown that a longitudinal stretching force F results in a reduced denaturation temperature $T_m(F)$, corresponding to force-induced destabilization of DNA. For the loop exponent in the presence of a finite $F > 0$ we found $c = 4\nu - 1/2 = 1.85$, so that the denaturation transition remains second order, but with an increased exponent. It would be interesting to study how the value of c is modified when self-avoiding interactions between a loop and the rest of the chain are included [13, 16].

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[22] One may study the interplay between $(\beta F a)^2$ and $\beta F a$ explicitly in a simplified model in which bound segments are always aligned along the x-direction. This produces a quadratic equation for f_m with t as a parameter, and $f_m(t)$ is a combination of both branches of the solution.

[23] This relies on the assumption that $p_\ell(\mathbf{r})$ is *Gaussian* for ideal random loops and as described above Eq. (7) for self-avoiding loops. For very large βF denatured loops are stretched out and aligned along F so that the partition function is dominated by parameter values for which $p_\ell(\mathbf{r})$ deviates from this form. A suitable $p_\ell(\mathbf{r})$ should be used to obtain the phase diagram in this regime.

[24] As such the potential $\ln Z(z, \omega, F)$ depends only on intensive parameters and would vanish due to the Euler equation. This is avoided by formally including the system volume $\mathcal{V}$ in the potential. Here $\mathcal{V} = \infty$ is understood.